A COMPARISON OF TWO METHODS OF INOCULATING *TRITICUM AESTIVUM* WITH SPORE SUSPENSIONS OF *CLAVICEPS PURPUREA*

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It has long been known that *Claviceps purpurea* (Fr.) Tul. can attack many species of grasses; Smith (1841), Carruthers (1874) and Moore (1959) all give lists of the British grasses most liable to attack. There are, however, conflicting reports concerning the ability of this fungus to pass from one host species to another. Stäger (1903, 1905, 1922) carried out an extensive series of cross-inoculations, covering many (but by no means all) of the possible combinations between twenty-eight species of grasses and isolates of *C. purpurea* from many of them. On the basis that infection failed to occur in many of the host/parasite combinations tested, Stäger concluded that this fungus is divided into several 'biologic forms' (or formae speciales as they would now be called), each with its own host range. More recently, Campbell (1957) reported the results of cross-inoculation experiments which were just as extensive as those of Stäger but more methodical in approach. He used in some experiments 421 conidial samples isolated from thirty-eight different host species to inoculate *Secale cereale* (Prolific), *Triticum aestivum* (Thatcher) and *Hordeum vulgare* (Newal) plants growing in the greenhouse, and in other experiments honeydew from *Secale cereale* to inoculate forty-six grass species growing in the field as well as in the greenhouse. Campbell obtained positive results with almost every combination of host and fungal isolate tested and therefore rejected Stäger's conclusions, suggesting that the latter's inoculation techniques may have been inadequate.

Before a flower of a susceptible grass can become infected with *C. purpurea* inoculum of the fungus must come into close proximity with the ovary. In nature, conidia or ascospores are transported by wind or insects to the ovary when the host is flowering and the glumes are open, and then germinate into mycelium. After penetrating the ovary wall near its base, the hyphae permeate the ovary which becomes progressively replaced by a mass of fungal tissue. There are various methods of infecting flowers experimentally, but they may be divided into two groups: (1) 'natural' methods which are comparable with the way in which infection takes place in nature, and (2) 'unnatural' methods to which there is no parallel process known in nature. Stäger (1903) used three methods of inoculation: dipping the heads into an inoculum of spores suspended in water, spraying the heads with the inoculum, and spraying the inoculum into the florets while the glumes were held apart. Campbell (1957) used four methods of inoculation: dipping the heads into a spore suspension, spraying the

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heads with a spore suspension, cutting off the tips of the glumes before spraying the inoculum into the florets, and injecting the inoculum into the floral cavities with a hypodermic syringe. The first two of these methods were used by Stäger, the third (i.e. clipping the tips of the glumes) is a more drastic version of Stäger's technique of holding the glumes apart to facilitate entry of the inoculum into the florets, while the fourth was used only by Campbell. Whereas all three methods used by Stäger can be classified as 'natural', the last two used by Campbell are clearly 'unnatural' in that the florets are damaged in the process of being inoculated.

Some of Stäger's conclusions are based on negative results from tests using only one of his methods of inoculation. If Campbell failed to obtain infection using one method, he repeated the inoculation using another method. It is impossible from the data in Campbell's paper to know which method was used in obtaining any particular result, but it can be inferred from his discussion that he found dipping the heads into the inoculum to be successful with only a few species, and that he often used the 'unnatural' methods of clipping the glumes or injecting with a hypodermic syringe. He states that Stäger might possibly have obtained many more positive results if he had tried an alternative method of inoculation whenever a negative result was obtained.

Against the background of the conflicting results of Stäger and Campbell, this article describes a simple experiment to find out to what extent the technique of inoculation may determine whether or not a potential host can become infected with *C. purpurea.*

Spore suspensions were obtained from isolates of *C. purpurea* growing on nine different host species (Table 1) and inoculated onto wheat (*Triticum aestivum* cv. Kolibri) by the two following methods. First, a spore suspension was sprayed with an atomizer onto inflorescences when the glumes were open (a 'natural' method), and secondly, each floret of an inflorescence was injected with a spore suspension using a hypodermic syringe (an 'unnatural' method).

The spore suspension was obtained as follows. A sterile slice of sclerotium from each host was plated on potato dextrose agar to establish a culture. After one week's growth at 20°, when the culture was actively growing, pieces of mycelium were transferred to 250 ml. Erlenmeyer flasks containing 50 ml of malt extract liquid medium. After ten days' growth on a shaker profuse sporulation had occurred, and a conidial suspension was obtained by filtering off the mycelium. This was diluted with sterile distilled water to a standard concentration of approximately 3000 spores/ml, and the resulting conidial suspension used as the inoculum. This concentration was chosen because a preliminary experiment, in which spore suspensions (isolated from *Triticum aestivum* and *Nardus stricta*) ranging from 50 spores/ml to 4000 spores/ml were tested, had indicated that 3000 spores/ml represented a suitable level of inoculum potential.

About ten heads of *T. aestivum* were inoculated with spores from each isolate, and a control set of heads was treated (sprayed or injected accord-

Table 1. *Production of ergots on* Triticum aestivum *after inoculation with isolates of* Claviceps purpurea *from different hosts, using two contrasting methods of inoculation*

Source of inoculum		Number of heads inoculated		Mean number of ergots per head	
Tribe	Species	Injected	Sprayed	Injected	Sprayed
Chlorideae	*Spartina anglica*	10	10	0	0
Nardeae	*Nardus stricta*	8	12	1·3	0
Glycerieae	*Glyceria fluitans*	13	10	3·2	0
Hordeae	*Triticum aestivum*	9	14	3·9	1·1
Festuceae	*Lolium perenne*	10	10	0·9	0·1
Brachypodieae	*Brachypodium sylvaticum*	10	11	0·1	0
Phalarideae	*Anthoxanthum odoratum*	10	10	0·1	0
Aveneae	*Arrhenatherum elatius*	10	10	0·4	0·2
	Deschampsia caespitosa	10	10	0·3	0
	Sterile distilled water (Control)	10	10	0	0

ing to the method being used) with sterile distilled water. The inoculations by injection were made when each head was just emerging from the leaf sheath, and each floret was injected with approximately 0·1 ml of the spore suspension. Sprayings were carried out during flowering time on two consecutive days to allow the total quantity of inoculum to be approximately the same as that injected into the florets. Infection was considered to have taken place when honeydew could be seen. If infection was not evident three weeks after the first spraying or injection, the treatment was repeated on another group of heads.

Table 1 shows that, when the test plants were injected with a spore suspension, eight of the nine treatments were successful but that, when they were sprayed, only three out of the nine were successful. The number of ergots that subsequently developed on infected heads also varied according to the isolate used, but it is interesting to note that there were more ergots per head when test plants were inoculated with an isolate from *T. aestivum* than with any of the other isolates. This applied to both the spraying and injecting methods. It will also be noticed that, in the three cases where both methods of inoculation produced infection, the injecting method gave a higher yield of ergot per head.

These results suggest that the method of inoculation influences both the presence and degree of infection. By using the injecting method wheat could be readily infected, whereas with the spraying method it would seem that certain isolates are prevented from achieving infection by some form of natural barrier. On the basis of these results the authors agree with Campbell that the technique of inoculation is important in determining whether or not infection with *C. purpurea* occurs, but they disagree with his assumption that a grass which can be infected under experimental conditions, no matter how drastic the inoculation technique used, will necessarily be infected in nature. They suggest that Stäger's conclusions are valid and may reflect the situation in nature more closely than those of Campbell.

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WHEAT SEED INFECTION BY *SEPTORIA TRITICI*

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In south-west England *Septoria tritici* Rob. ex Desm., the cause of wheat speckled leaf blotch, is commonly found on winter wheat seedlings but the inoculum source for these early infections is not fully established. Infected wheat debris has been implicated as a possible inoculum source for seedling infection (Jovićević, 1960) but Williams & Jones (1973) have observed *S. tritici* early in the season in wheat crops following non-cereal crops. The high incidence of the head phase of *S. tritici* during the 1971–2 season in south-west England stimulated interest in the possible rôle of infected wheat seed in the epidemiology of the pathogen. The head phase was first reported by Luthra, Sattar & Ghani (1937) in India and later observed by Arsenijević (1965) in Yugoslavia. In Britain, it was not until the 1968–9 season that Jones & Cooke (1970) observed head symptoms on the winter wheat cv. Champlein in Pembrokeshire, Wales. However, previous attempts to isolate the pathogen from seed harvested from infected plants have failed (Luthra, Sattar & Ghani, 1938; Williams, 1972). The work reported here concerns the production of artificially infected seed with the subsequent detection of the pathogen in the seed using an agar plate technique, the effect of two surface sterilizing agents on the mycelium in the seed, and the effect of an organo-mercurial seed treatment on the mycelial growth of *S. tritici* in culture.

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